Agriculture, Ecosystems and Environment 74 (1999) 77–93

Agriculture Ecosystems & Environment

# Biodiversity of arbuscular mycorrhizal fungi in agroecosystems

David D. Douds Jr. [a,*], Patricia D. Millner [b]

[a] *USDA-ARS ERRC, 600 E. Mermaid Lane, Wyndmoor, PA, 19038 USA*

[b] *USDA-ARS Soil Microbial Systems Laboratory, Building 001, BARC-West, Beltsville, MD, 20705 USA*

**Abstract**

Arbuscular mycorrhizal fungi [AMF] are soil fungi which form a mutualistic symbiosis with the roots of plants. A variety of benefits to the host have been ascribed to mycorrhizae, most often enhanced uptake of immobile nutrients from the soil, notably P. An understanding of the impacts of agronomic practices upon communities of these fungi would help to ensure an opportunity for the utilization of the symbiosis and contribute to the success of sustainable agriculture. Measurement of the diversity of AMF communities in field soils presents a variety of challenges, among them the difficulty of identifying field collected spores, the detection of non-sporulating members of the community, and the lack of relation between functional diversity and the morphological diversity of spores used to delineate species. These challenges, as well as current efforts to overcome them, are discussed and recent research elaborating the effects of agromonic practices upon AMF communities and the effectiveness of mycorrhizae is presented. ©Elsevier Science B.V.

*Keywords:* Arbuscular mycorrhizal fungi; Communities; Diversity; Techniques

## 1. Introduction

Arbuscular mycorrhizal fungi [AMF] are obligately symbiotic soil fungi which colonize the roots of the majority of plants. These fungi are so named because they produce characteristic finely branched hyphal structures, termed arbuscules, inside cortical cells of plant roots. The AMF genera *Gigaspora* and *Scutellospora* produce only arbuscules and inter- and intracellular hyphae, whereas *Glomus*, *Entrophospora*, *Acaulospora*, and *Sclerocystis* also produce vesicles (hence the frequently used term vesicular-arbuscular mycorrhizal [VAM] fungi), which are terminal, globose, lipid rich structures in intracellular areas of the root cortex (Strullu et al., 1983). Fossil evidence suggests this association dates back to when plants first grew on land, some 400 million years ago (Remy et al., 1994) and indeed, it is the non-mycorrhizal plant that is the rarity in nature.

The AM symbiosis is typically mutualistic. As obligate symbionts, AMF are believed to be dependent upon the host plant for fixed carbon. The plant receives a variety of benefits which may result in increased growth: improved water relations (Davies et al., 1993), pest and disease resistance (Hooker et al., 1994), enhanced nutrient uptake over non-mycorrhizal controls (George et al., 1995), and modification of root morphology (Berta et al., 1990). The most important of these benefits is increased nutrient uptake, notably of immobile nutrients such as P and Zn (Bolan, 1991; Bürkert and Robson, 1994). Extra-radical hyphae of the AMF extend up to 8 cm beyond the root (Rhodes and Gerdemann, 1975) and act, in effect, as extensions of the root system in acquiring nutrients from the soil.

* Corresponding author. Tel.: +1-215-233-6421; fax: +1-215-233-6581
*E-mail address:* ddouds@arserrc.gov (D.D. Douds Jr.)



PII: S0167-8809(99)00031-6

The below-ground ecosystem as a whole is affected by AMF. These fungi are important in maintaining and enhancing the stability of soil aggregates (Tisdall and Oades, 1979; Miller and Jastrow, 1990, 1992). Soil aggregation is an important aspect of soil structure, which determines characteristics such as water inflow rate, pore space, and resistance to erosion. Extra-radical AMF hyphae enmesh and entrap soil particles, stabilizing the aggregates. Members of the Gigasporineae may play a greater role in this than those of the Glomineae (Miller and Jastrow, 1992; Schreiner and Bethlenfalvay, 1995). Functioning extra-radical hyphae also secrete a glycoprotein termed glomalin (Wright et al., 1996). This substance may be present at levels as high as 1.5% of the dry weight of soil (Wright and Upadhyaya, 1996) and there is evidence it plays a significant role in the production and maintenance of water stable aggregates in soil. In addition, AMF substantially affect nutrient cycling (Jeffries and Barea, 1994) and carbon flow from the autotrophic plant to the heterotrophic soil microbial community because of their effect on root exudation (Graham, 1985). This regulation of carbon flow can be an important regulator of the soil microbial community (Linderman, 1991). For example, the presence of *Glomus mosseae* affected the relative abundance of rhizosphere bacteria species (Ames et al., 1984).

It is evident from their effects upon soil health and host plant growth that AMF are an important part of sustainable agricultural systems that have low inputs of chemical fertilizers and biocides (Bethlenfalvay and Schüepp, 1994; Jeffries and Barea, 1994; Hooker and Black, 1995). Modern, intensive agricultural practices, such as chemical fertilization and pest control, continuous monoculture, and tillage impact AMF and plant interactions. Describing the diversity of the community of AMF at a site becomes, therefore, an important step in determining the effects of agricultural treatments upon AMF and the eventual development of management regimes for these fungi.

## 2. Methods for the study of AMF

### 2.1. Isolation of spores from soil

There are several methods to isolate spores of AMF from field soil. These include wet sieving and decanting (Gerdemann and Nicolson, 1963; Vilarino and Arines, 1990), flotation-adhesion (Sutton and Barron, 1972), airstream fractionation (Tommerup, 1982), several different water/sucrose centrifugation methods (Ianson and Allen, 1986), and fixing soil slurries to filter paper (Smith and Skipper, 1979; Khalil et al., 1994).

Perhaps the most commonly used method combines wet sieving and decanting with a method adapted from nematology (Jenkins, 1964). The procedure begins with the soil sample being stirred vigorously in water (e.g., 1–2 l for a 50 $cm^3$ sample). Sodium hexametaphosphate may be added to help disperse soil particles and aid release of AMF spores (McKenney and Lindsey, 1987). The liquid is then poured onto a nest of sieves (425 μm pore size on top to allow passage of spores but retain large soil and organic matter particles, and 25–40 μm on the bottom to catch all but the smallest AMF spores yet allow passage of the finest soil particles). Material in the smallest sieve is washed into a centrifuge tube and centrifuged (4 min at $1000\times g$). The supernatant is then discarded and the pellet resuspended in 40% (w/v) sucrose and centrifuged again for 1 min. Spores and other organisms such as nematodes and mites remain in the supernatant, which is poured onto a sieve, rinsed with water, and washed onto a Petri plate for initial observation and collection of spores (Millner, 1987). This limited exposure to sucrose infrequently results in osmotic damage to the spores, but, in such cases, Renografin 60 (Furlan et al., 1980) or silica (Verkade, 1988) may be used instead of sucrose in the final centrifugation.

### 2.2. Quantfication of AMF colonization of roots

The most common way to examine AMF colonization of roots is through the dissecting microscope (20–25×) after clearing roots in 10% KOH (w/v) followed by staining in trypan blue (Phillips and Hayman, 1970). Chlorazol black E (Brundrett et al., 1984) and acid fuchsin (Merryweather and Fitter, 1991) also have been used to stain these fungi in root tissue. Heavily pigmented roots may require harsher clearing treatments in addition to KOH, for example heating in a mixture of $NH_4OH$ and $H_2O_2$ (Koske and Gemma, 1989).

Quantification of fungal colonization is accomplished by microscopic examination of stained roots or via quantification of fungus-specific compounds in the roots. Microscopic measurement utilizing the gridline intersect method (Newman, 1966; Giovannetti and Mosse, 1980) has an added advantage in that it also allows for the simultaneous determination of root length. A variation of the gridline intersect method, developed by McGonigle et al. (1990b), is used at 160–200× to determine the proportion of root length in which arbuscles, vesicles or hyphae occur.

The most common chemical method for quantification of AMF colonization of roots is the determination of chitin (Ride and Drysdale, 1972; Bethlenfalvay et al., 1981), present in the cell walls of AMF. Chitin is also present in a variety of other fungi and insects, so visual estimates of colonization usually are conducted concurrently to guard against fungal contamination and estimate intensity of infection (Bethlenfalvay and Pacovsky, 1981). Quantification of other compounds, such as campesterol, 24-methylenecholesterol (Schmitz et al., 1991), ergosterol (Frey et al., 1994), and yellow carotenoid pigments found in some mycorrhizal roots (Bothe et al., 1994) has been correlated with degree of AMF colonization.

### *2.3. Identification of AMF*

Characterization of species-level diversity may provide the necessary basic information needed to assess the impact of soil and crop management practices on AMF communities and indirectly on some of the soil biological aspects of sustainable agricultural practices. However, for AMF, species level comparisons only provide an assessment of morphological diversity because the life-history traits important to the functional symbiosis (e.g., amount and architecture of external hyphae; proportional fungal biomass as arbuscules versus vesicles, hyphae, auxillary cells, or spores; and absorptive and transport capacity of hyphae) are not linked to any apparent character traits used to distinguish species (Morton and Bentivenga, 1994). Indeed, individual isolates of AMF species can differ so much that some may be considered as plant growth promoters, whereas others are borderline pathogens (Modjo and Hendrix, 1986; Sylvia et al., 1993). Identification and its appropriate application and interpretation is further challenged by the obligate dependence of these fungi on plant hosts; they cannot be cultured axenically like other fungi or bacteria. This has impeded the rapid development of a wide array of identification tools as has occurred with other microbes.

Spore characters and their various states of expression, especially spore wall structure (i.e., number of layers, size, color, refractivity, flexibility, histologic reactivity, ornamentation, etc.) and developmental sequence remain the basis for species-level taxonomy (Franke and Morton, 1994; Morton and Bentivenga, 1994). The costs and difficulties encountered in publishing and updating illustrated guides that include keys, species descriptions, terminology, definitions, and citations have motivated several groups of researchers to develop computer-based identification aids as an alternative to hard-copy versions previously used (Walker, 1983; Walker and Sanders, 1986; Schenck and Perez, 1990). One easily accessible and reliable source of information to aid with identification of AMF species can be found at the 150 page World Wide Web site with the URL address: http://invam.caf.wvu.edu/ established by J. Morton and S. Bentivenga of INVAM (the International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi, University of West Virginia, Department of Plant and Soil Sciences, Morgantown, West Virginia, USA). This site is the repository of the most current information available on taxonomy, morphology, spore characteristics, culture, and preservation methods for this group of plant symbionts. All the reference isolates included in the compilations and descriptions are based on direct links to the type materials, a link which has heretofore not been consistently available to general users. This 'type'-linked basis is a valuable advance beyond the primarily literature-based keys previously available (Hall and Fish, 1979; Trappe, 1982). A second aid is available on CD-ROM through BEG (Bank of European Glomales, a European counterpart to INVAM), which can be contacted on the World Wide Web at their URL address: http://kiwi.ukc.ac.uk/biolab/beg/index.html.

The reader is referred to the URL site above for information on species descriptions. The brief characteristics that follow include the major distinguishing traits for identification to the suborder level. Several morphological characters within the order Glomales distinguish members of the suborder Glomineae (which includes *Glomus*, *Acaulospora*, *Entrophospora*, and *Sclerocystis*) from members of the suborder Gigasporineae (including *Gigaspora* and *Scutellospora*). The presence of a conspicuous swelling directly below the spore on the spore-bearing hypha (also known as a bulbous suspensor-like cell), a permanent outer layer of the spore wall laminae, and production of auxillary cells external to the root characterize Gigasporineae taxa. *Gigaspora* are distinguished from *Scutellospora* by the presence of a discrete structure known as the germination shield on the inner spore wall. In contrast, the production of vesicles in roots is characteristic of Glomineae taxa, but not Gigasporineae. Few such readily observable distinctions occur between the genera of the Glomineae, and those at the species level are often even more subtle. For example, the main distinction between *Acaulospora* and *Entrophospora* is the location of the spore bud (lateral or within the neck) relative to the neck of the preformed saccule. After this event, Morton (1995) states that “discrete successional stages in spore differentiation are identical in species of both genera.” The precise reason for maintaining the genus *Sclerocystis* for an individual species is not entirely clear at present. A considerable amount of discussion has left unresolved the issue of just how different the single species, *S. coremioides*, is from other taxa now classified as *Glomus* species.

## 3. Sampling

### 3.1. Where

Though spores of AMF have been found in soil as deep as 2.2 m (Zajicek et al., 1986), 70–85% of spores are found in the top 40–45 cm (Jakobsen and Nielsen, 1983; Thompson, 1991). It is necessary to sample more than the surface 15 cm because some species have been shown to be more abundant deeper in the soil profile (An et al., 1990a; Douds et al., 1995).

### 3.2. When

Spores of AMF show seasonal patterns of abundance in natural environments. Spore populations typically are greatest in the autumn in areas where there are marked warm/cold seasons (Douds and Chaney, 1982; Gemma and Koske, 1988; Dhillon and Anderson, 1993; Klironomos et al., 1993). Populations are typically at their lowest during the growing season (An and Hendrix, 1993) or, in tropical climates, during the monsoon season (Singh and Varma, 1981). A further advantage in collecting spores in the autumn, soon after they are produced, is that spores will be in better condition for identification. Some species, such as *Gigaspora gigantea* (Gemma and Koske, 1988), *Glomus clarum* (Louis and Lim, 1988), *Acaulospora longula* (Douds and Schenck, 1991), and others (Tommerup, 1983) exhibit dormancy, so spores of these species collected in autumn should be refrigerated for several months rather than used immediately to initiate pot cultures (see below).

### 3.3. How many

Spores of AMF may occur in clumped distributions in the field (St. John and Koske, 1988), not correlated to root distribution (Friese and Koske, 1991). Up to 30 samples were needed to recover all species found in a sand dune soil (Tews and Koske, 1986) because of this heterogeneity. Only seven samples were needed to find all sporulating AMF species in an agricultural soil in southeastern Pennsylvania, USA (Franke-Snyder, Galvez and Douds, unpublished).

## 4. Special considerations in determining diversity of AMF

Inoculum of AMF exists in soil in three forms: spores, soilborne hyphae and colonized roots. The taxonomic identification of AMF species is based upon morphological characteristics of their spores (size, color, and spore wall characteristics; Schenck and Perez, 1990; Morton and Bentivenga, 1994). Hyphae of an infection unit of one AMF only infrequently can be distinguished microscopically from another inside a root (Abbott, 1982), and then typically only when one knows the identity of the two fungi and is familiar with their characteristics from prior observation in single species culture (Abbott and Gazey, 1994). The large diameter and lack of septae and clamp connections distinguish some soil hyphae of AMF from those of other fungi (Sylvia, 1992), whereas the fine mycorrhizal hyphae are not easily distinguished from those of other soil fungi. Antibodies to glomalin, a recently described glycoprotein produced and abundantly shed by AMF, can be used to distinguish AMF hyphae from non-AMF hyphae in soil (Wright et al., 1996). Even so, mycorrhizal hyphae in experimental situations are usually quantified by counting all hyphae in inoculated soils and subtracting hyphal lengths found in uninoculated, control soils (Sylvia, 1992) with no breakdown by species in mixed AMF inocula. Distinctions among AMF hyphae can usually only be made microscopically at the family level and above (Morton and Bentivenga, 1994).

Immunochemical methods also have been applied to the identification and study of AM fungi (Perotto et al., 1994; Hahn et al., 1994). Significant success has been achieved with monoclonal antibodies, even to the point of discriminating between isolates of a species (Wright et al., 1987). However, even monoclonal antibodies may show significant cross reactivity. Of 22 hybridomas producing antibodies to *Glomus mosseae*, 20 cross reacted with *Glomus occultum* and *Acaulospora spinosa* (Cebulla et al., 1994). Low percentages of hybridomas producing antibodies to AMF antigens have been a problem in applying this technology to AMF (Hahn et al., 1993). Immunochemical methods have not been applied to communities of AMF in the field to date.

Spores and intraradical vesicles of AMF are composed primarily of lipids (Bielby and Kidby, 1980; Jabaji-Hare et al., 1984). Further, some of the fatty acids present are unique, and may be utilized to distinguish AMF lipids from host lipids and one AMF species from another (Jabaji-Hare, 1988; Graham et al., 1995). For example, $\Delta 11$ hexadecenoic acid was found in spores, hyphae, and roots colonized by *Glomus mosseae*, but not in uncolonized roots of *Allium porrum* (Sancholle and Dalpé, 1994; Grandmougin-Ferjani et al., 1995). Differences in profiles of fatty acids among AMF indicate that fatty acid methyl ester [FAME] analysis may be useful to identify individual species of AMF (Graham et al., 1995). This procedure has utility for pure samples of individual species, but may not be able to identify those species present in a root or soil sample containing an unknown number of species.

The molecular techniques of polymerase chain reaction [PCR] and restriction fragment length polymorphism [RFLP] have been applied to the identification AM fungi (Abbott and Gazey, 1994; diBonoto et al., 1995). Most success has been achieved by amplifying the nuclear DNA encoding for the small subunit rRNA and the attendant internal transcribed regions known to vary at the species level. Nevertheless, some of these probes have only family or genus level specificity (Simon et al., 1993). Application of this technique to field collected spores can be frustrating because the yield of PCR products may be 'variable and unpredictable' (Sanders et al., 1995). The application of this technique to ten *Glomus* spores collected from the field, all of similiar size and color, yielded ten different banding patterns after digestion with two restriction endonucleases (Sanders et al., 1995). Classical taxonomy was not conducted on these spores to determine if they were the same or different species, but this method may be impractical if it yields a 'too-reductionist' view of an AMF community. There is evidence that this method may detect differences between populations of nuclei within a spore (Sanders et al., 1995), further complicating this method's utility in field situations.

Therefore, one is faced with the formidable task of making taxon specific probes to all species in a natural AMF community. This would be quite formidable in the case of soil from a field with a sorghum–soybean rotation in Nebraska, USA, in which 26 species of AMF were found (Ellis et al., 1992). The classical method of spore identification is the more viable alternative, presently. There are several issues which must be considered when traditional taxonomic identification of spores is used to describe AMF community diversity. Firstly, the relative abundance of spores of a species may not reflect its functional importance or even its relative biomass contribution to the community as a whole, i.e., the number of spores in the soil may not reflect the relative amount of colonization of roots by this fungus or the amount and distribution of hyphae in the soil. Secondly, non-sporulating species may be present (Miller et al., 1985; Clapp et al., 1995). A fungus may be a significant member of the 'vegetative' community, but, because of date of sampling, local environment, or host plant regulation of carbon expenditure, be unable to produce spores yet be well able to persist to the following year as infective hyphae in roots or soil. Thirdly, spores collected from the field may be difficult to identify as a result of degradation of spore walls (Morton, 1993).

Several approaches can be used to address these issues. Molecular methods (discussed above) can be used to detect taxa or taxonomic groupings of AMF in roots or soil. In addition, non-sporulating species can often be coaxed to sporulate in ‘trap cultures.’ Plants are potted in the greenhouse, typically in a 1 : 1 (v/v) mixture of field soil and sand, and the potting mix is sampled at intervals for spore production. Sometimes two or three cycles of trap cultures are needed before spores of some species appear (Morton et al., 1995; Stutz and Morton, 1996). In addition, plants from the field can be removed to the greenhouse and transplanted into soil free of AMF to encourage sporulation of species colonizing that plant. All these methods were needed to detect the 23 species present in a grassland (Bever et al., 1996). Roots collected from the field will also harbor non-sporulating species and should also be used to inoculate trap cultures.

Appearance of a species in a trap culture gives no indication of that species’ abundance in the originial field population, however. An approach to this problem is a variation of the Most Probable Number (MPN) method (An et al., 1990b) normally used to determine the concentration of AMF propagules or colony-forming units of bacteria or fungi in inoculum (Porter, 1979). Plants are grown in a series of dilutions of field soil mixed with sterilized soil. The soil is sampled for AMF spores after 9 weeks of growth in the greenhouse. Mathematical tables are consulted after noting the highest dilution in which a spore of a species is produced, and calculations yield the number of propagules of that species. However, the developmental progression from propagules to colonization to sporulation is not the same for each species or growth condition, so these estimates may not accurately reflect the abundance of all species in the original samples. This method does provide the advantage of detecting species not found when isolating spores directly from soil (An et al., 1990b).

The problem of poor condition of field collected spores is remedied through the production of greenhouse ’pot cultures.’ Single, healthy, field collected spores, or groups of spores that are likely to be the same species, are inoculated onto the root systems of seedlings in the greenhouse. Graminaceous host plants typically are used, such as bahiagrass (*Paspalum notatum* Flugge) or sorghum–sudangrass (*Sorghum bicolor*(L.) Moench. × *S. sudanense* (Piper) Stapf.). The potting mix is typically a pasteurized/autoclaved mixture of native field soil and sand. Cultures are monitored for sporulation by routine sampling. Spores produced in pot culture are in a condition more conducive to identification than the original spores collected from the field. However, not all species can be cultured routinely. Some cultures will be unsuccessful because of failure of spores to germinate, germination but failure to colonize the roots, or colonization but no sporulation under the growing conditions used. For example, only 2 of 12 species of AMF from sand dune soils in Brazil were successfully cultured (Stürmer and Bellei, 1994). These data, as well as those from the trap cultures, yield information only about the species present at the field site, not their relative abundance. A compromise to this problem is the identification of field collected AMF spores to ‘species type groups’ to enable some calculation of relative abundance (Dodd et al., 1990; Douds et al., 1993).

An additional challenge is that potentially very few of the existing AMF species have been described (Morton et al., 1995). Thus, spores obtained from field soils may not be from any of the 150 or so species currently described (see Section 6).

Given these complications in the study of AMF communities at field sites, the following long-term strategy may be employed. Preliminary thorough sampling of the study site is conducted: spores are isolated, segregated into species type groups, and pot cultures initiated. Trap cultures are initiated using soil and roots collected from the field. Both types of cultures are monitored regularly for spore production. Spores are isolated, identified, and single species pot cultures are produced. Species richness of the site then can be calculated. Once an overall familiarity with the AMF species of the site is achieved, the potential for identification of spores directly from field soil in response to management practices exists. Dominance, diversity and biovolume indices may then be calculated to describe the sporulating community.

## 5. Impacts of soil management and cropping system practices

Numerous greenhouse studies have shown the effects of various chemical, physical, and biotic variables upon AMF and the development of mycorrhizae. Among experimental treatments which affect various aspects of the development of mycorrhizae are nutrient levels, soil disturbance, and host plant species. This work is being transferred to the field to quantify the effects of chemical addition (conventional versus low-input agriculture), tillage, and crop rotations upon indigenous populations of AMF in an effort to manage for these beneficial fungi.

Chemical additions, notably of P in greenhouse experiments, depress both colonization of roots and sporulation by the majority of AMF (Allen et al., 1981; Douds and Schenck, 1990). A parallel is seen in field settings where lower levels of mycorrhizal fungus spores, inoculum potentials, and colonization of crop plants are seen in conventional, chemical-based agriculture versus low-input, alternative agriculture (Limonard and Ruissen, 1989; Kurle et al., 1991; Vivekanandan and Fixen, 1991; Douds et al., 1993, 1995; Kurle and Pfleger, 1994; Ryan et al., 1994). The farming system also affects the species makeup of the AMF community (see Section 6).

The extra-radical phase of the AMF functions as the nutrient absorbing organ of the mycorrhiza and inoculum for colonization of new roots. Both of these functions are adversely affected by soil disturbance in greenhouse and growth chamber studies (Fairchild and Miller, 1990; McGonigle et al., 1990a). Field studies utilizing mechanical or hand tillage have confirmed these findings (Vivekanandan and Fixen, 1991; McGonigle and Miller, 1993; Galvez et al., 1995). Tillage may also affect the structure of the indigenous community of AMF (see Section 6).

Greenhouse pot culture experiments have revealed optimal host-fungus combinations from the perspective of both enhancement of plant growth (Pope et al., 1983) and production of AMF spores (Struble and Skipper, 1985; Hetrick and Bloom, 1986; Bever et al., 1996). Crop plant host and crop rotation are also known to affect AMF in the field. Continuous monocultures can both decrease populations of AMF spores (Rao et al., 1995) and shift the AMF species composition of the community toward species which may not be beneficial to the crop (Fyson and Oaks, 1991; Johnson et al., 1992) (See Section 6). The presence of non-mycotrophic plants, such as members of the Cruciferae, decreases the inoculum of AMF (Harinikumar and Bagyaraj, 1988) as do long periods of bare ground fallow (Thompson, 1987). Crop rotations also affect species diversity (see Section 6).

## 6. Case histories of AMF in field environments

Diversity of AMF has been studied in a variety of natural and agricultural ecosystems (Table 1). Due to the difficulties outlined above, the characteristic measured most often is the species richness of a site. Fourteen species, two previously undescribed, were found in the rhizospheres of plants in a bamboo forest in Taiwan (Wu and Chen, 1986). Thirteen species were found in an old meadow site in Que., Canada, with the number of species isolated per sample increasing with conversion to barley cultivation (Hamel et al., 1994). *Glomus constrictum* spores were 22–38% of the total. The trap culture technique was used to produce spores for identification from six sites under cultivation in Sask., Canada (Talukdar and Germida, 1993). Only three to six species were found at each site, with *Glomus mosseae* and *Glomus clarum* found at all six sites. As mentioned earlier, 26 species were found at a site with a *S. bicolor–Glycine max* rotation in Nebraska, USA (Ellis et al., 1992). Soil samples were collected over 6 years to describe the AMF community across Poland (Blaszkowski, 1993). Thirty-seven species, six unidentified, were found in cultivated soils, with *Glomus deserticola* occurring most frequently and, overall, the most dominant species. Forty-six species, ten previously undescribed, were found in natural areas. *Glomus constrictum* was the most frequently occurring species and was most dominant by a wide margin. Thirty-four species were found in soils on the Hel Peninsula, Poland, with *Scutellospora dipurpurascens* occurring most frequently (Blaszkowski, 1994). *Glomus macrocarpum* and *Glomus etunicatum* were the most frequently occurring of the 22 most abundant species in an area of the Everglades National Park, FL, USA, undergoing restoration (Aziz et al., 1995). Twenty-three AMF species were found in a mown grassland site in North Carolina, USA, seven of which previously were undescribed (Bever et al.,

Table 1
A summary of numbers of AMF species found in natural and managed ecosystems

| Environment | Species No. | Trap cultures used | References |
|---|---|---|---|
| Bamboo Forest, Taiwan | 14 | – | Wu and Chen, 1986 |
| Old meadow, Quebec | 13 | – | Hamel et al., 1994 |
| Grassland, North Carolina, USA | 23 | + | Bever et al., 1996 |
| Tallgrass prairie, Kansas, USA | 14 | – | Bentivenga and Hetrick, 1992 |
| Sonoran desertscrub, Arizona, USA | 7–9 | + | Stutz and Morton, 1996 |
| Sand dune, Rhode Island, USA | 6 | – | Koske and Halvorson, 1981 |
| Sao Paulo, Brazil | 19 | – | Trufem, 1995 |
| Santa Catarina, Brazil | 12 | – | Stürmer and Bellei, 1994 |
| Sandy soil, Hel Peninsula, Poland | 34 | – | Blaszkowski, 1994 |
| Native vegetation, Poland | 46 | – | Blaszkowski, 1993 |
| Cultivated soils | 37 | – | |
| Native woodland, Florida, USA | 10 | + | Schenck and Kinloch, 1980 |
| Cultivated | 9–12 | + | |
| *Malus domestica* orchards, USA | 3–10 | + | Miller et al., 1985 |
| Conventional agriculture | | | |
| Saskatchewan | 3–6 | + | Talukdar and Germida, 1993 |
| *G. max* and *Z. mays*, Minnesota, USA | 12 | – | Johnson et al., 1991 |
| *G. max* Kentucky, USA | 13 | + | An et al., 1993b |
| *Festuca arundinacea*, Kentucky, USA | 16 | + | An et al., 1993b |
| *Digitaria sanguinalis*, Kentucky, USA | 13 | + | Guo et al., 1993 |
| *Pennisetum americanum*, Kentuck | 14 | + | Guo et al., 1993 |
| *S. bicolor*, *G. max*, Nebraska, USA | 26 | - | Ellis et al., 1992 |
| *Z. Mays*, Maryland, USA | 20 | + | Watson and Millner (unpublished) |
| Low-input, *Z. mays/G. max*, Minnesota, USA | 13 | + | Kurle and Pfleger, 1996 |
| Conventional | 10 | + | |
| Low-input, *Z. mays/G. max*, Pennsylvania | 13 | + | Franke-Snyder and Douds (unpublished) |
| Conventional | 12 | + | |

1996). Twelve species were found in dune soil in Santa Catarina, Brazil, with *Acaulospora scrobiculata* most abundant and *Gigaspora albida* occurring in each sample (Stürmer and Bellei, 1994). This community was similiar to those of sand dunes in the eastern USA (Koske, 1987). Up to 19 species were found in sand dune soils in Brazil (Trufem, 1995). Spores of the Gigasporaceae were more common in Brazilian dune soils, whereas the Glomaceae or Acalosporaceae were more common in the wet tropical forest of Brazil (Trufem, 1990).

The effects of agricultural practices upon AMF communities have been studied by several groups in North America, building upon the pioneering work of (Kruckelmann, 1975). These investigations will be presented as case histories.

### 6.1. *The effects of crop rotation upon AMF communities—University of Kentucky, USA*

The effects of crop rotation upon AMF were studied primarily in relation to the control of the tobacco stunt disease pathogen, *Glomus macrocarpum* (Hendrix et al., 1992). The principle of certain hosts being less conducive to the reproduction of particular AMF was applied to this unique situation in which growth supression is caused by an AMF. Tall fescue (*Festuca arundinacea*) in rotation decreased populations of *G. macrocarpum* below those detrimental to tobacco, whereas sorghum–sudangrass increased populations of *G. macrocarpum* (An et al., 1993a; Hendrix et al., 1995).

The effects of other crop rotations upon AMF communities also have been studied by this group. The AMF communities were described for portions of a field after either 3 years of soybean or 2 years of fescue (An et al., 1990a). Field soil from the fescue plots had 6× as many spores as soil from continuous soybean. The MPN assays found 5× as many propagules with fescue versus soybean. In addition, the MPN/trap culture method (An et al., 1990b) yielded 13 species in the continuous soybean plots versus 16 species in the fescue. Work with other rotations showed *Glomus* spp.

prevailing in rotation but *Gigaspora* spp. more numerous in continuous soybeans (An et al., 1993b). The continuous soybean plots had lower species richness and diversity but higher dominance and equitability indices versus plots planted to maize, milo (*Sorghum bicolor*(L.) Moench), or fescue. However, after a crop of soybean was grown in all plots, these differences disappeared, indicating the AMF community characterized after crop harvest in autumn reflects primarily the effects of that crop and not previous cropping history. Change in crop host can cause some AMF to decline to levels undetectable by trap culture methods, or cause others to increase from previously undetectable levels (Guo et al., 1993).

### *6.2. The effects of soil disturbance/tillage upon mycorrhizae and nutrient absorption of maize—University of Guelph, Ont., Canada*

Since the extra-radical network of mycelium of AMF functions as both the nutrient absorbing organ of the mycorrhiza and as inoculum for the colonization of new roots, it follows that soil disturbance from tillage may affect both colonization by AMF of plants sown into this soil and mycorrhizae-mediated nutrient uptake. This phenomenon has been examined in field and growth chamber studies by researchers at the University of Guelph (Miller et al., 1995).

A series of growth chamber experiments showed that maize plants grown in disturbed soil were less colonized by AMF and had lower shoot P and Zn concentrations than plants grown in undisturbed, field collected soil (Evans and Miller, 1988; Fairchild and Miller, 1988, Fairchild and Miller, 1990). No effect of soil disturbance was seen for spinach (*Spinacea oleracea*) and rape (*Brassica napus*), two species which are not colonized by AMF (Evans and Miller, 1988). In addition, application of the fungicide benomyl to disturbed and undisturbed pots decreased the effect of disturbance by lessening the P uptake by mycorrhizae in undisturbed soils (see also Kunishi and Bandel, 1991). These experiments suggest that a new seedling is benefited by a previously established AMF hyphal network in the soil (Evans and Miller, 1990). In addition, if the availability of P in the soil were high enough to preclude any benefit from mycorrhizae, there should be no effect of soil disturbance on the P concentration of plants. This is what was found (Fairchild and Miller, 1990), even though mycorrhiza formation was greater on plants in undisturbed soil. Zinc absorption by plants was inhibited by disturbance in this experiment independent of P nutrition, as expected because of inhibition of formation of mycorrhizae from soil disturbance.

Results of the growth chamber studies were verified in the field. Maize grown in no-till or ridge tillage management exhibited greater early season P absorption and mycorrhizal colonization than plants grown in moldboard plowed soils (McGonigle et al., 1990a; McGonigle and Miller, 1993, 1996). Although this shows that less P is needed in reduced tillage farm management, something as yet undetermined causes no-tilled maize to lose its early growth advantage and yield no better than plants in tilled soils (Miller et al., 1995).

### *6.3. The effects of fertilization and continuous monocultures upon the structure/function of AMF communities—University of Minnesota, USA*

Studies in fields planted to continuous maize or continuous soybean for 5 years showed the effect of crop host and local edaphic factors upon the relative abundance of spores of AMF (Johnson et al., 1991). Twelve species were isolated directly from field soil at each of two sites. The spore population of site 2 was overwhelmingly dominated by *Glomus aggregatum* (90% of the community, but only 10% at site 1), giving site 1 greater evenness and a higher Shannon Weiner diversity index (1.59 for site 1 versus 0.42 for site 2). Some species were found to be more abundant in continuous maize than in soybean, e.g., *Glomus aggregatum*, *Glomus leptotichum*, and *Glomus occultum* at site 1. Others were more numerous with soybeans, e.g., *Glomus microcarpum* at site 1.

The AMF that become numerous with continuous monocultures may contribute to the yield declines over time noted for such crops (Johnson et al., 1992). Plots with a history of continuous maize were strip planted to either maize or soybeans. Those with a history of soybeans were treated likewise. Spores of AMF species which proliferated on maize (*G. mosseae*, *G. occultum*, *G. aggregatum* and *G. leptotichum*) were negatively correlated with the yield of the following maize crop, but positively correlated with the yield of the subsequent soybean crop. The relationship was less clear for spores proliferating on soybean.

The difference in AMF communities in response to different soil–crop managment practices raises the hypothesis of whether those AMF abundant in high nutrient, well-fertilized soils are plant growth promoters or just more aggressive than other AMF at acquiring host carbon for their own reproduction. Eight years of fertilization of low nutrient soil caused populations of four species, including *Gigaspora gigantea*, to decline and *Glomus intraradices* to increase (Johnson, 1993). This confirmed other work which showed *G. gigantea* more associated with natural or low-input systems than in conventional agriculture (Miller and Jastrow, 1992; Douds et al., 1993) and that *G. intraradices* is very tolerant of high nutrient situations (Sylvia and Schenck, 1983). Communities from fertilized or unfertilized plots were collected and inoculated onto big bluestem grass (*Andropogon gerardi*) grown in the greenhouse. After 1 month of growth, plants inoculated with the 'unfertilized community' were larger than those inoculated with the 'fertilized community' (Johnson, 1993). Microscopic observation of the mycorrhizas suggested AMF from the fertilized community may have been a greater carbon drain on their host plants relative to AMF from the unfertilized community. The former produced the same proportion of root length with vesicles as those from the unfertilized community, but a lower proportion of root length which had arbuscules, the site of nutrient transfer to the host. Interestingly, other isolates of *G. intraradices* are beneficial to the growth of plants (Graham and Timmer, 1985; Hamel et al., 1992).

### *6.4. The effects of farming system and crop rotation on AMF—Rodale Institute Research Center and US Department of Agriculture, Pennsylvania, USA*

The AMF populations in soils managed via conventional, chemical based agriculture have been contrasted to those in soils under a variety of low-input agriculture management regimes. Low-input agriculture, as developed and practiced at the Rodale Institute Research Center, consists of diverse crop rotations involving legume and other overwintering cover crops to supply and retain soil N, compete with weeds, and retard soil erosion. Several characteristics of these different farming systems contribute to the observed greater levels of spores of AMF and colonization of plants in soil managed with low-input methods versus conventional chemical inputs (Douds et al., 1993, 1995) (Table 2). One major difference is the absence of chemical fertilizer, notably P, in the low-input system. Phosphorus fertilizer is widely known to inhibit colonization of roots and spore production by AMF (Allen et al., 1981; Douds and Schenck, 1990). The second major difference is that fields in the low-input system, as described above, have live plant cover for a greater proportion of an average year than fields with a conventional maize/soybean rotation (Douds et al., 1993). This is primarily the result of overwintering cover crops in the low-input system. An overwintering cover crop of hairy vetch (*Vicia villosa*) was shown to increase the inoculum of AMF in soil (Galvez et al., 1995). In addition, bare soil was 10°C warmer than soil from plots with the cover crop in late spring (Galvez et al., 1995). It is detrimental to AMF spores and hyphae to be in warm, moist soil with no host roots present (Nemec, 1987; Douds and Schenck, 1991). The warmth and moisture, and $CO_2$ generated by the concurrently stimulated microbial activity, encourage spore germination and hyphal growth (Bécard and Piché, 1989; Poulin et al., 1993) in the absence of suitable hosts. This expends energy and leaves the inoculum less vigorous for the eventual colonization of crop plant roots.

Other aspects of agricultural practices, such as tillage and crop rotations discussed in other case histories, also have been examined at this site. Plants from seed sown into no-tilled soils were more colonized by AMF than those in tilled soil (Galvez et al., 1995). Tillage affected the distribution of spores and inoculum and affected the whole-soil profile populations of spores of different spore type groups (Douds et al., 1995).

Crop rotation clearly affected spore populations of AMF in another crop–soil management trial utilizing a maize–vegetable–small grain rotation and chemical fertilizer or organic amendments as sources of mineral nutrients. Two general conclusions could be drawn: (1) pepper (*Capsicum annuum*), a plant which averaged less than 2% root length colonized throughout the growing season, depressed populations of AMF spores (Table 3) and levels of inoculum (Table 4) and (2) different species type groups sporulated more heavily

Table 2
Summary of results of autumn sampling for AMF spores at the Rodale Institute Research Center Farming Systems Trial, 1989–1994[a]

| Farming System | *Glomus* spp.[b] | *Glomus occultum* group[c] | *Gigaspora gigantea* |
|---|---|---|---|
| Low-input with animal manure | $12.6 \pm 2.1$ | $46.7 \pm 8.6$ | $2.9 \pm 1.2$ |
| Low-input cash grain[d] | $15.3 \pm 1.9$ | $57.1 \pm 16.6$ | $7.2 \pm 2.5$ |
| Conventional | $5.6 \pm 0.8$ | $21.9 \pm 2.3$ | $0.3 \pm 0.1$ |

[a] Spores per 50 $cm^3$ soil. Data are summed across crop rotation through the years, yielding high SEMs.
[b] Other *Glomus*species, including *Glomus mosseae*.
[c] Small hyaline *Glomus*spores, < 100 μm diameter.
[d] Legume cover crops for nitrogen.

Table 3
Effect of previous crop upon populations of AM fungus spores. Soil was collected on 4 December, 1995[a]

| Crop | Spore type group | | | | |
|---|---|---|---|---|---|
| | *Gigaspora gigantea* | *Glomus occultum* group[b] | *Glomus*spp. (other)[c] | *Glomus etunicatum* group[d] | *Glomus* sp. A[e] |
| Pepper | 0.5 a | 19.0 b | 4.5 b | 6.8 a | 1.3 b |
| Maize | 0.6 a | 28.6 ab | 24.0 a | 10.3 a | 11.2 a |
| Wheat | 0.8 a | 35.4 a | 21.2 a | 13.1 a | 10.1 a |

[a]Spores per 50 $cm^3$, means of 48 observations. Numbers in the same column followed by the same letter are not significantly different ($\alpha = 0.05$, Tukey's Method of Multiple Comparisons).
[b]Small hyaline *Glomus*spores, <100 μm diameter.
[c]Other *Glomus*species, including *Glomus mosseae*.
[d]Small, yellow *Glomus*spores, approximately 100 μm diameter, also includes *Glomus intraradices.*
[e]Large hyaline *Glomus*spores, 150–200 μm diameter.

Table 4
Results of infectivity assays conducted in the growth chamber with 1 : 1 (v/v) mixtures of field soil and vermiculite[a]

| Rotation | Colonization of test plant | |
|---|---|---|
| | 14 June, 1995 | 4 December, 1995 |
| Pepper | 20.5 b | 8.2 b |
| Maize | 20.8 b | 22.8 a |
| Wheat | 28.6 a | 19.5 a |

[a] *Paspalum notatum* as test plant for 6/14 and *Sorghum bicolor* × *Sorghum sudanese* for 12/4. Means of 36 observations, numbers in the same column followed by the same letter are not significantly different ($\alpha = 0.05$), Tukey's Method of Multiple Comparisons.

on one crop than on others. For example, *G. gigantea* (Fig. 1(A)) and the *Glomus etunicatum* type group (Fig. 1(B)) sporulated more heavily after a maize crop whereas the *Glomus occultum* type group (Fig. 1(C)) produced more spores when a small grain (*Triticum aestivum* or *Hordeum vulgare*) was the last crop.

## 7. Conclusion

A complete description of the AMF community of a soil would include the identity of fungi present as spores, hyphae in the soil, and intraradical hyphae and vesicles as well as information on the relative abundance of each species in each component. Such an exhaustive description is impossible at present. Molecular techniques exist to identify hyphae in roots and soil of a few of the 155 described AMF species. Though these methods show great promise, currently they have utility only to trace the persistence of an introduced isolate for which one has a probe, or interactions among several isolates under controlled conditions. Quantification of the effects of agricultural management practices upon communities of AMF presently requires a compromise. Only total hyphal length in soil and total mycorrhization of host roots can be quantified. Identification of species at a site and quantification of diversity, dominance, etc., presently is limited to the sporulating species. Non-sporulating species can be detected via trap culture techniques which give no indication of the species' relative abundance in the original sample. A description of the community that is based on spore counts and identification probably reflects inaccurately the total contributed biomass of each species to the community. Furthermore, it provides no informa-

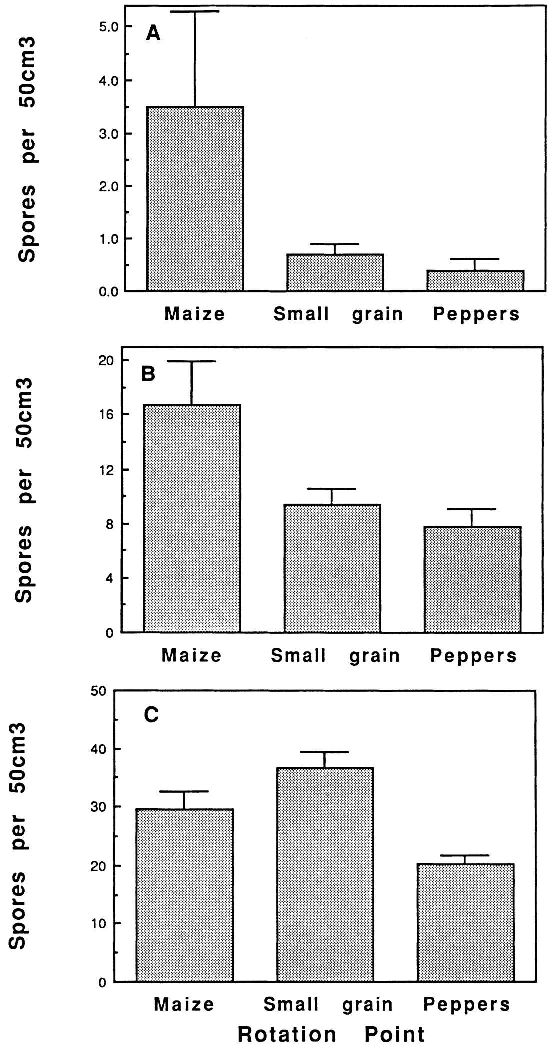


Fig. 1. Populations of spores of (A) *Gigaspora gigantea*, (B) the *Glomus etunicatum* type group (also includes *Glomus intraradices* and an undescribed *Glomus*), and (C) the *Glomus occultum* type group. Bars are the means ± SEM for 3 years' replication of each crop host.

tion about which species are primary contributors of the extra-radical hyphae which enhance nutrient uptake of roots and produce glomalin, the glycoprotein with a significant role in soil aggregation.

## References


Abbott, L.K., 1982. Comparative anatomy of vesicular-arbuscular mycorrhizas formed on subterranean clover. Aust. J. Bot. 30, 485–499.

Abbott, L.K., Gazey, C., 1994. An ecological view of the formation of AM mycorrhizas. Plant Soil 159, 69–78.

Allen, M.F., Sexton, J.C., Moore, T.S. J Jr., Christensen, M., 1981. Influence of phosphate source on vesicular-arbuscular mycorrhizae of *Bouteloua gracilis*. New Phytol. 87, 687–694.

Ames, R.N., Reid, C.P.P., Ingham, E.R., 1984. Rhizosphere bacterial population responses to root colonization by a vesicular-mycorrhizal fungus. New Phytol. 96, 555–563.

An, Z.-Q., Hendrix, J.W., 1993. Populations of spores and propagules of mycorrhizal fungi in relation to the life cycles of tall fescue and tobacco. Soil Biol. Biochem. 25, 813–817.

An, Z.-Q., Grove, J.H., Hendrix, J.W., Hershman, D.E., Henson, G.T., 1990a. Vertical distribution of endogonaceous mycorrhizal fungi associated with soybean as affected by soil fumigation. Soil Biol. Biochem. 22, 715–719.

An, Z.-Q., Hendrix, J.W., Hershman, D.E., Henson, G.T., 1990b. Evaluation of the most probable number (MPN) and wet-sieving methods for determining soil-borne populations of Endogonaceous mycorrhizal fungi. Mycologia 82, 576–581.

An, Z.-Q., Guo, B.Z., Hendrix, J.W., 1993a. Mycorrhizal pathogen of tobacco: cropping history and current crop effects on the mycorrhizal fungal community. Crop Protection 12, 527–531.

An, Z.-Q., Hendrix, J.W., Hershman, D.E., Ferriss, R.S., Henson, G.T., 1993b. The influence of crop rotation and soil fumigation on a mycorrhizal fungal community associated with soybean. Mycorrhiza 3, 171–182.

Aziz, T., Sylvia, D.M., Doren, R.F., 1995. Activity and species composition of arbuscular mycorrhizal fungi following soil removal. Ecol. Appl. 5, 775–784.

Bécard, G., Piché, Y., 1989. Fungal growth stimulation by $CO_2$ and root exudates in vesicular-arbuscular mycorrhizal symbiosis. Appl. Environ. Microbiol. 55, 2320–2325.

Bentivenga, S.P., Hetrick, B.A.D., 1992. The effect of prairie management practices on mycorrhizal symbiosis. Mycologia 84, 522–527.

Bielby, J.P., Kidby, D.K., 1980. Biochemistry of ungerminated and germinated spores of the vesicular-arbuscular mycorrhizal fungus, *Glomus caledonius*: changes in neutral and polar lipids. J. Lipid Res. 21, 739–750.

Berta, G., Fusconi, A., Trotta, A., Scannerini, S., 1990. Morphogenetic modifications induced by the mycorrhizal fungus strain E3 in the root system of *Allium porrum* L. New Phytol. 114, 207–215.

Bethlenfalvay, G.J., Pacovsky, R.S., 1981. Growth inhibition and enhancement in soybeans by *Glomus fasciculatus*. In: Proc. 5th North American Conf. on Mycorrhizae, 16–21 August, 1981, Que., Canada, 12 pp.

Bethlenfalvay, G.J., Pacovsky, R.S., Brown, M.S., 1981. Measurement of mycorrhizal infection in soybeans. Soil Sci. Soc. Am. J. 45, 871–875.

Bethlenfalvay, G.J., Schüepp, H., 1994. Arbuscular mycorrhizas and agrosystem stability. In: Gianinazzi, S., Schüepp, H. (Eds.), Impact of Arbuscular Mycorrhizas on Sustainable Agriculture and Natural Ecosystems. Birkhauser Verlag; Basel, Switzerland, pp. 117–131.

Bever, J.D., Morton, J.B., Antonovics, J., Schultz, P.A., 1996. Host-dependent sporulation and species diversity of arbuscular mycorrhizal fungi in a mown grassland. J. Ecol. 84, 71–82.

Blaszkowski, J., 1993. Comparative studies of the occurrence of arbuscular fungi and mycorrhizae (Glomales) in cultivated and uncultivated soils of Poland. Acta Mycologia 28, 93–140.

Blaszkowski, J., 1994. Arbuscular fungi and mycorrhizae (Glomales) of the Hel Peninsula, Poland. Mycorrhiza 5, 71–88.

Bolan, N.S., 1991. A critical review of the role of mycorrhizal fungi in the uptake of phosphorus by plants. Plant Soil 134, 189–208.

Bothe, H., Klinger, A., Kaldorf, M., Schmitz, O., Esch, H., Hundeshagen, B., Kernebeck, H., 1994. Biochemical approaches to the study of plant–fungal interactions in arbuscular mycorrhiza. Experientia 50, 919–925.

Brundrett, M.C., Piché, Y., Peterson, R.L., 1984. A new method for observing the morphology of vesicular-arbuscular mycorrhizae. Can. J. Bot. 62, 2128–2134.

Bürkert, B., Robson, A., 1994. $^{65}$Zn uptkake in subterranean clover (*Trifolium subterraneum* L.) by three vesicular-arbuscular mycorrhizal fungi in a root-free sandy soil. Soil Biol. Biochem. 26, 1117–1124.

Cebulla, G.D., Strobel, R., Kurle, J.E., Pfleger, F.L., 1994. Production of monoclonal antibodies to selected arbuscular mycorrhizal fungi. Proc. Annual Meeting of the Am. Phytopathol. Soc., 6–10 August. Poster, 668 pp.

Clapp, J.P., Young, J.P., Merryweather, J.W., Fitter, A.H., 1995. Diversity of fungal symbionts in arbuscular mycorrhizae from a natural community. New Phytol. 130, 259–265.

Davies, F.T., Porter, J.R., Linderman, R.G., 1993. Drought resistance of mycorrhizal pepper plants—independent of leaf phosphorus concentration, response in gas exchange, and water relations. Physiol. Plant 87, 45–53.

Dhillon, S.S., Anderson, R.C., 1993. Seasonal dynamics of dominant species of arbuscular mycorrhizae in burned and unburned sand prairies. Can. J. Bot. 71, 1625–1630.

diBonoto, R., Elliott, M.L., des Jardin, E.A., 1995. Detection of an arbuscular mycorrhizal fungus in roots of different plant species with the PCR. Appl. Environ. Microbiol. 61, 2809–2810.

Dodd, J.C., Arias, I., Kooman, I., Hayman, D.S., 1990. The management of populations of vesicular-arbuscular mycorrhizal fungi in acid-infertile soils of a savanna ecosystem II. The effects of pre-crops on the spore populations of native and introduced VAM fungi. Plant Soil 122, 241–248.

Douds, D.D., Chaney, W.R., 1982. Correlation of fungal morphology and development to host growth in a green ash mycorrhiza. New Phytol. 92, 519–526.

Douds, D.D., Schenck, N.C., 1990. Increased sporulation of vesicular-arbuscular mycorrhizal fungi by manipulation of nutrient regimes. Appl. Environ. Microbiol. 56, 413–418.

Douds, D.D., Schenck, N.C., 1991. Germination and hyphal growth of VAM fungi during and after storage in soil at five matric potentials. Soil Biol. Biochem. 23, 177–183.

Douds, D.D., Janke, R.R., Peters, S.E., 1993. VAM fungus spore populations and colonization of roots of maize and soybean under conventional and low-input sustainable agriculture. Agric. Ecosys. Environ. 43, 325–335.

Douds, D.D., Galvez, L., Jank, R.R., Wagoner, P., 1995. Effect of tillage and farming system upon populations and distribution of vesicular-arbuscular mycorrhizal fungi. Agric. Ecosys. Environ. 52, 111–118.

Ellis, J.R., Roder, W., Mason, S.C., 1992. Grain sorghum–soybean rotation and fertilization influence on vesicular-arbuscular mycorrhizal fungi. Soil Sci. Soc. Am. J. 56, 783–794.

Evans, D.G., Miller, M.H., 1988. Vesicular-arbuscular mycorrhizas and the soil-disturbance-induced reduction of nutrient absorption in maize I. Causal relations. New Phytol. 110, 67–74.

Evans, D.G., Miller, M.H., 1990. The role of the external mycelial network in the effect of soil disturbance upon vesicular-arbuscular mycorrhizal colonization of maize. New Phytol. 114, 65–72.

Fairchild, G.L., Miller, M.H., 1988. Vesicular-arbuscular mycorrhizas and the soil-disturbance-induced reduction of nutrient absorption in maize II. Development of the effect. New Phytol. 110, 75–84.

Fairchild, G.L., Miller, M.H., 1990. Vesicular-arbuscular mycorrhizas and the soil-disturbance-induced reduction of nutrient absorption in maize III. Influence of phosphorus amendments to soil. New Phytol. 114, 641–650.

Franke, M., Morton, J.B., 1994. Ontogenetic comparisons of arbuscular mycorrhizal fungi *Scutellospora heterogama* and *Scutellospora pellucida*: revision of taxonomic character concepts, species descriptions and phylogenetic hypothesis. Can. J. Bot. 72, 122–134.

Frey, B., Vilarino, A., Schuepp, M., Arines, J., 1994. Chitin and ergosterol content of extraradical and intraradical mycelium of the vesicular-arbuscular mycorrhizal fungus *Glomus intraradices*. Soil Biol. Biochem. 26, 711–717.

Friese, C.F., Koske, R.E., 1991. The spatial dispersion of spores of vesicular-arbuscular mycorrhizal fungi in a sand dune microscale: patterns associated with the root architecture of American beachgrass. Mycol. Res. 95, 952–957.

Furlan, V., Bartschi, H., Fortin, J.-A., 1980. Media for density gradient extraction of endomycorrhizal spores. Trans. Br. Mycol. Soc. 75, 336–338.

Fyson, A., Oaks, A., 1991. Promotion of maize growth by legume soil factors. In: Keister, D.L., Cregan, P.B. (Eds.), The Rhizosphere and Plant Growth. Kluwer Academic Publishers, The Netherlands, 370 pp.

Galvez, L., Douds, D.D., Wagoner, P., Longnecker, L.R., Drinkwater, L., Janke, R.R., 1995. An overwintering cover crop increases inoculum of VAM fungi in agricultural soil. Am. J. Alternative Agric. 10, 152–156.

Gemma, J.N., Koske, R.E., 1988. Seasonal variation in spore abundance and dormancy of *Gigaspora gagantea* and in mycorrhizal inoculum potential of a dune soil. Mycologia 80, 211–216.

George, E., Marschner, H., Jakobsen, I., 1995. Role of arbuscular mycorrhizal fungi in uptake of phosphorus and nitrogen from soil. Crit. Rev. Biotechnol. 15, 257–270.

Gerdemann, J.W., Nicolson, T.H., 1963. Spores of mycorrhizal Endogone species extracted by wet sieving and decanting. Trans. Br. Mycol. Soc. 46, 235–244.

Giovannetti, M., Mosse, B., 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytol. 84, 489–500.

Graham, J.H., 1985. Membrane regulation of mycorrhizal symbiosis. In: Proc. 6th North American Conf. on Mycorrhizae. 25–29 June, 1984. Bend, OR, USA, pp. 155–156.

Graham, J.H., Timmer, L.W., 1985. Rock phosphate as a source of phosphorus for vesicular-arbuscular mycorrhizal development and growth of citrus in a soilless medium. J. Am. Soc. Hortic. Sci. 110, 489–492.

Graham, J.H., Hodge, N.C., Morton, J.B., 1995. Fatty acid methyl ester profiles for characterization of Glomalean fungi and their endomycorrhizae. Appl. Environ. Microbiol. 61, 58–64.

Grandmougin-Ferjani, A., Dalpé, Y., Veignie, E., Hartmann, M.A., Rafin, C., Sancholle, M., 1995. Infection by arbuscular mycorrhizal fungus *Glomus mosseae* of leek plants (*Allium porrum* L.): effect on lipids. In: Kader, J.-C., Mazliak, P. (Eds.), Plant Lipid Metabolism. Kluwer Academic Publishers, The Netherlands, pp. 444–446.

Guo, B.Z., An, Z.-Q., Hendrix, J.W., Dougherty, C.T., 1993. Influence of a change from tall fescue to pearl millet or crabgrass on the mycorrhizal fungal community. Soil Sci. 155, 393–405.

Hahn, A., Bonfante, P., Horn, K., Pausch, F., Hock, B., 1993. Production of monoclonal antibodies against surface antigens of spores from arbuscular mycorrhizal fungi by an improved immunization and screening procedure. Mycorrhiza 4, 69–78.

Hahn, A., Gianinazzi-Pearson, V., Hock, B., 1994. Characterization of arbuscular mycorrhizal fungi by immunochemical methods. In: Gianinazzi, S., Schüepp, H. (Eds.), Impact of Arbuscular Mycorrhizas on Sustainable Agriculture and Natural Ecosystems. Birkhauser Verlag, Basel, Switzerland, pp. 25–39.

Hall, I.R., Fish, B.J., 1979. A key to the Endogonaceae. Trans. Br. Mycol. Soc. 73, 261–270.

Hamel, C., Furlan, V., Smith, D.L., 1992. Mycorrhizal effects on interspecific plant competition and nitrogen transfer in legume–grass mixtures. Crop Sci. 32, 991–996.

Hamel, C., Dalpé, Y., Lapierre, C., Simard, R.R., Smith, D.L., 1994. Composition of the vesicular-arbuscular mycorrhizal fungus population in an old meadow as affected by pH, phosphorus and soil disturbance. Agric. Ecosys. Environ. 49, 223–231.

Harinikumar, K.M., Bagyaraj, D.J., 1988. Effect of crop rotation on native vesicular-arbuscular mycorrhizal propagules in soil. Plant Soil 110, 77–80.

Hendrix, J.W., Jones, K.J., Nesmith, W.C., 1992. Control of pathogenic mycorrhizal fungi in maintenance of soil productivity by crop rotation. J. Prod. Agric. 5, 383–386.

Hendrix, J.W., Guo, B.Z., An, Z.-Q., 1995. Divergence of mycorrhizal communities in crop production systems. Plant Soil 170, 131–140.

Hetrick, B.A.D., Bloom, J., 1986. The influence of host plant on production and colonization ability of vesicular-arbuscular mycorrhizal spores. Mycologia 78, 32–36.

Hooker, J.E., Black, K.E., 1995. Arbuscular mycorrhizal fungi as components of sustainable soil–plant systems. Crit. Rev. Biotechnol. 15, 201–212.

Hooker, J.E., Jaizme-Vega, M., Atkinson, D., 1994. Biocontrol of plant pathogens using arbuscular mycorrhizal fungi. In: Gianinazzi, S., Schüepp, H. (Eds.), Impact of Arbuscular Mycorrhizas on Sustainable Agriculture and Natural Ecosystems. Birkhäuser Verlag, Basel, Switzerland, pp. 191–200.

Ianson, D.C., Allen, M.F., 1986. The effects of soil texture on extraction of vesicular-arbuscular mycorrhizal spores from arid soils. Mycologia 78, 164–168.

Jabaji-Hare, S., 1988. Lipid and fatty acid profiles of some vesicular-arbuscular mycorrhizal fungi: contribution to taxonomy. Mycologia 80, 622–629.

Jabaji-Hare, S., Deschene, A., Kendrick, B., 1984. Lipid content and composition of vesicles of a vesicular-arbuscular mycorrhizal fungus. Mycologia 76, 1024–1030.

Jakobsen, I., Nielsen, N.E., 1983. Vesicular-arbuscular mycorrhiza in field-grown crops I. Mycorrhizal infection in cereals and peas at various times and soil depths. New Phytol. 93, 401–413.

Jeffries, P., Barea, J.M., 1994. Biogeochemical cycling and arbuscular mycorrhizas in the sustainability of plant–soil systems. In: Gianinazzi, S., Schüepp, H. (Eds.), Impact of Arbuscular Mycorrhizas on Sustainable Agriculture and Natural Ecosystems. Birkhäuser Verlag, Basel, Switzerland, pp. 101–115.

Jenkins, W.R., 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Dis. Rep. 73, 288–300.

Johnson, N.C., 1993. Can fertilization of soil select less mutualistic mycorrhizae?. Ecol. Appl. 3, 749–757.

Johnson, N.C., Pfleger, F.L., Crookston, R.K., Simmons, S.R., 1991. Vesicular-arbuscular mycorrhizas respond to corn and soybean cropping history. New Phytol. 117, 657–664.

Johnson, N.C., Copeland, P.J., Crookston, R.K., Pfleger, F.L., 1992. Mycorrhizae: possible explanation for yield decline with continuous corn and soybean. Agron. J. 84, 387–390.

Khalil, S., Carpenter-Boggs, L., Loynachan, T.E., 1994. Procedure for rapid recovery of VAM fungal spores from soil. Soil Biol. Biochem. 26, 1587–1588.

Klironomos, J.N., Moutogolis, P., Kendrick, B., Widden, P., 1993. A comparison of spatial heterogeneity of vesicular-arbuscular mycorrhizal fungi in two maple-forest soils. Can. J. Bot. 71, 1472–1480.

Koske, R.E., 1987. Distribution of VA mycorrhizal fungi along a latitudnal temperature gradient. Mycologia 79, 55–68.

Koske, R.E., Halvorson, W.L., 1981. Ecological studies of vesicular-arbuscuar mycorrhizae in a barrier sand dune. Can. J. Bot. 59, 1413–1422.

Koske, R.E., Gemma, J.N., 1989. A modified procedure for staining roots to detect VA mycorrhizas. Mycol. Res. 92, 486–488.

Kruckelmann, H.W., 1975. Effects of fertilizers, soils, soil tillage and plant species on the frequency of Endogone chlamydospores and mycorrhizal infection in arable soils. In: Sanders, F.E., Mosse, B., Tinker, P.B. (Eds.), Endomycorrhizas. Academic Press, London, pp. 511–525.

Kunishi, H.M., Bandel, V.A., 1991. Microbe enhanced P uptake by corn under no-till and conventional till. In: Keister, D.L., Cregan, P.B. (Eds.), The Rhizosphere and Plant Growth. Kluwer Academic Publishers, The Netherlands, 368 pp.

Kurle, J.E., Pfleger, F.L., 1994. Arbuscular mycorrhizal fungus spore populations respond to conversions between low-input and conventional management practices in a corn–soybean rotation. Agron. J. 86, 467–475.

Kurle, J.E., Pfleger, F.L., 1996. Management influences on arbuscular mycorrhizal fungal species composition in a corn–soybean rotation. Agron. J. 88, 155–161.

Kurle, J.E., Pfleger, F.L., Crookston, R.K., 1991. The effect of management and edaphic factors on vesicular-arbuscular mycorrhizal (VAM) populations in a corn–soybean rotation. Phytopathology 81, 1210.

Limonard, T., Ruissen, M.A., 1989. The significance of VA-mycorrhiza to future arable farming in The Netherlands. Netherlands J. Plant Pathol. 95I Suppl. 1, 129–136.

Linderman, R.G., 1991. Mycorrhizal interactions in the rhizosphere. In: Keister, D.L., Cregan, P.B. (Eds.), The Rhizosphere and Plant Growth. Kluwer Academic Publishers, The Netherlands, pp. 343–348.

Louis, I., Lim, G., 1988. Effect of storage of inoculum on spore germination of a tropical isolate of *Glomus clarum*. Mycologia 80, 157–161.

McGonigle, T.P., Miller, M.H., 1993. Does the more effective mycorrhizal symbiosis found with reduced tillage increase corn yield? 1993 Annual Report, Land Resource Science. University of Guelph, Guelph, Ont., Canada, pp. 69–70.

McGonigle, T.P., Miller, M.H., 1996. Mycorrhizae, phosphorus absorption and yield of maize in response to tillage. Soil Sci. Soc. Am. J.s 60, 1856–1861.

McGonigle, T.P., Evans, D.G., Miller, M.H., 1990a. Effect of degree of soil disturbance on mycorrhizal colonization and phosphorus absorption by maize in growth chamber and field experiments. New Phytol. 116, 629–636.

McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, G.L., Swan, J.A., 1990b. A new method which gives an objective measure of colonization of roots by vesicular-mycorrhizal fungi. New Phytol. 115, 495–501.

McKenney, M.C., Lindsey, D.L., 1987. Improved method for quantifying endomycorrhizal fungi spores from soil. New Phytol. 79, 779–782.

Merryweather, J.W., Fitter, A.H., 1991. A modified method for elucidating the structure of the fungal partner in a vesicular-arbuscular mycorrhiza. Mycol. Res. 95, 1435–1437.

Miller, R.M., Jastrow, J.D., 1990. Hierarchy of root and mycorrhizal fungal interactions with soil aggregation. Soil Biol. Biochem. 22, 579–584.

Miller, R.M., Jastrow, J.D., 1992. The role of mycorrhizal fungi in soil conservation. In: Bethlenfalvay, G.J., Linderman, R.G. (Eds.), Mycorrhizae in Sustainable Agriculture. Agron. Soc. Am. Special Publication No. 54. Madison, WI, pp. 24–44.

Miller, D.D., Domoto, P.A., Walker, C., 1985. Mycorrhizal fungi at 18 apple rootstock plantings in the United States. New Phytol. 100, 379–391.

Miller, M.H., McGonigle, T.P., Addy, H.D., 1995. Functional ecology of vesicular-arbuscular mycorrhizas as influenced by phosphate fertilization and tillage in an agricultural ecosystem. Crit. Rev. Biotechnol. 15, 241–255.

Millner, P.D., 1987. A spore collection apparatus for vesicular-arbuscular mycorrhizal fungi. Mycologia 79, 899–900.

Modjo, H.S., Hendrix, J.W., 1986. The mycorrhizal fungus *Glomus macrocarpum* as a cause of tobacco stunt disease. Phytopathology 76, 688–691.

Morton, J.B., 1993. Problems and solutions for the integration of glomalean taxonomy, systematic biology, and the study of mycorrhizal phenomena. Mycorrhiza 2, 97–109.

Morton, J., 1995. Mode of spore formation *Acaulospora* and *Entrophospora*: are differences great enough to justify generic distinctions? INVAM Newslett. 5(1), 7-8 (also available on Internet at http://invam.caf.wvu.edu/).

Morton, J.B., Bentivenga, S.P., 1994. Levels of diversity in endomycorhizal fungi (Glomales, Zygomycetes) and their role in defining taxonomic and non-taxonomic groups. Plant Soil 159, 47–59.

Morton, J.B., Bentivenga, S.P., Bever, J.D., 1995. Discovery, measurement, and interpretation of diversity in arbuscular endomycorrhizal fungi (Glomales, Zygomycetes). Can. J. Bot. 73 (Suppl.), s25–s32.

Nemec, S., 1987. Effect of storage temperature and moisture on Glomus species and their subsequent effect on citrus rootstock seedling growth and mycorrhiza development. Trans. Br. Mycol. Soc. 89, 205–212.

Newman, E.I., 1966. A method of estimating the total length of root in a sample. J. Appl. Ecol. 3, 139–145.

Perotto, S., Brewin, N.J., Bonfante, P., 1994. Colonization of pea roots by the mycorrhizal fungus *Glomus versiforme* and by *Rhyzobium* bacteria: immunological comparison using monoclonal antibodies for plant cell surface components. Mol. Plant–Microbe Interactions 7, 91–98.

Phillips, J.M., Hayman, D.S., 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55, 158–160.

Pope, P.E., Chaney, W.R., Woodhead, S., Rhodes, J.D., 1983. Vesicular-arbuscular mycorrhizal species influence the mycorhizal dependency of four hardwood tree species. Can. J. Bot. 61, 412–417.

Porter, W.M., 1979. The most probable number method for enumerating infective propagules of vesicular-arbuscular mycorrhizal fungi in soil. Aust. J. Soil Res. 17, 515–519.

Poulin, M.J., Bel Rhlid, R., Piché, Y., Chênevert, R., 1993. Flavonoids released by carrot (*Daucus carota*) seedlings stimulate hyphal development of vesicular-arbuscular mycorrhizal fungi in the presence of optimal $CO_2$ enrichment. J. Chem. Ecol. 19, 2317–2327.

Rao, A.V., Tarafdar, J.C., Sharma, S.K., Aggarwal, R.K., 1995. Influence of cropping systems on soil biochemical properties in an arid rain-fed environment. J. Arid Environ. 31, 237–244.

Remy, W., Taylor, T.N., Hass, H., Kerp, H., 1994. Four hundred million year old vesicular-arbuscular mycorrhizae. Proc. Natl. Acad. Sci. U.S.A. 91, 11841–11843.

Rhodes, L.H., Gerdemann, J.W., 1975. Phosphate uptake zones of mycorrhizal and non-mycorrhizal onions. New Phytol. 75, 555–561.

Ride, J.P., Drysdale, R.B., 1972. A rapid method for the chemical estimation of filamentous fungi in plant tissue. Physiol. Plant Pathol. 2, 7–15.

Ryan, M.H., Chilvers, G.A., Dumaresq, D.C., 1994. Colonization of wheat by VA-mycorrhizal fungi was found to be higher on a farm managed in an organic manner than on a conventional neighbor. Plant Soil 160, 33–40.

Sancholle, M., Dalpé, Y., 1994. Taxonomic relevance of fatty acids of arbuscular mycorrhizal fungi and related species. Mycotaxon 49, 187–193.

Sanders, I.R., Alt, M., Groppe, K., Boller, T., Wiemken, A., 1995. Identification of ribosomal DNA polymorphisms among and within spores of the Glomales: application to studies on the genetic diversity of arbuscular mycorrhizal fungal communities. New Phytol. 130, 419–427.

Schenck, N.C., Kinloch, R.A., 1980. Incidence of mycorrhizal fungi on six field crops in monoculture on a newly cleared woodland site. Mycologia 72, 445–456.

Schenck, N.C., Perez, Y., 1990. Manual for the Identification of VA Mycorrhizal Fungi, 3rd ed. Synergistic Publications, Gainesville, FL, USA, 241 pp.

Schmitz, O., Danneberg, G., Hundeshagen, B., Klinger, A., Bothe, H., 1991. Quantification of vesicular-arbuscular mycorrhiza by biochemical parameters. J. Plant Physiol. 139, 106–114.

Schreiner, R.P., Bethlenfalvay, G.J., 1995. Mycorrhizal interactions in sustainable agriculture. Crit. Rev. Biotechnol. 15, 271–285.

Simon, L., Levesque, R.C., Lalonde, M., 1993. Identification of endomycorrhizal fungi colonizing roots by fluorescent single-strand conformation polymorphism–polymerase chain reaction. Appl. Environ. Microbiol. 59, 4211–4215.

Singh, K., Varma, A.K., 1981. Endogonaceous spores associated with xerotrophic plants in northern India. Trans. Br. Mycol. Soc. 77, 655–658.

Smith, G.W., Skipper, H.D., 1979. Comparison of methods to extract spores of vesicular-arbuscular mycorrhizal fungi. Soil Sci. Soc. Am. J. 43, 722–725.

St. John, T.V., Koske, R.E., 1988. Statistical treatment of endogonaceous spore counts. Trans. Br. Mycol. Soc. 91, 117–121.

Struble, J.E., Skipper, H.D., 1985. Spore production by five VAM fungi as influenced by plant species. In: Proc. 6th North American Conf. on Mycorrhizae, 25–29 June, 1984, Bend, OR, USA, 251 pp.

Strullu, D.G., Elroy, J.F., Gourret, J.P., 1983. Ultrastructure and analysis, by laser probe mass spectroscopy of the mineral composition of the vesicles of *Trifolium pratense* endomycorrhizas. New Phytol. 94, 81–88.

Stürmer, S.L., Bellei, M.M., 1994. Compositional and seasonal variation of spore populations of arbuscular mycorrhizal fungi in dune soils on the island of Santa Catarina, Brazil. Can. J. Bot. 72, 359–363.

Stutz, J.C., Morton, J.B., 1996. Successive pot cultures reveal high species richness of arbuscular mycorrhizal fungi in arid soils. Can. J. Bot. 74, 1883–1889.

Sutton, J.C., Barron, G.L., 1972. Population dynamics of *Endogone* spores in soil. Can. J. Bot. 50, 1909–1914.

Sylvia, D.M., 1992. Quantification of external hyphae of vesicular-arbuscular mycorrhizal fungi. In: Norris, J.R., Read, D.J., Varma, A.K. (Eds.), Methods in Microbiology. 24: Techniques For the Study of Mycorrhiza. Academic Press, London, pp. 53–65.

Sylvia, D.M., Schenck, N.C., 1983. Application of superphosphate to mycorrhizal plants stimulates sporulation of phosphorus-tolerant vesicular-arbuscular mycorrhizal fungi. New Phytol. 95, 655–661.

Sylvia, D.M., Wilson, D.O., Graham, J.H., Maddox, J.J., Millner, P., Morton, J.B., Skipper, H.D., Wright, S.F., Jarstfer, A.G., 1993. Evaluation of vesicular-arbuscular mycorrhizal fungi in diverse plants and soils. Soil Biol. Biochem. 25, 705–713.

Talukdar, N.C., Germida, J.J., 1993. Occurrence and isolation of vesicular-arbuscular mycorrhizae in cropped field soils of Sask., Canada. Can. J. Microbiol. 39, 567–575.

Tews, L.L., Koske, R.E., 1986. Toward a sampling strategy for vesicular-arbuscular mycorrhizas. Trans. Br. Mycol. Soc. 87, 353–358.

Thompson, J.P., 1987. Decline of vesicular-arbuscular mycorrhizae in long fallow disorder of field crops and its expression in phosphorus deficiency of sunflower. Aust. J. Agric. Res. 38, 847–867.

Thompson, J.P., 1991. Improving the mycorrhizal condition of the soil through cultural practices and effects on growth and phosphorus uptake by plants. In: Johansen, C., Lee, K.K., Sahrawat, K.L. (Eds.), Phosphorus Nutrition of Grain Legumes in the Semi-Arid Tropics. Int. Crops Research Instit. for the Semi-arid Tropics, pp. 117–138.

Tisdall, J.M., Oades, J.M., 1979. Stabilization of soil aggregates by the root systems of ryegrass. Aust. J. Soil Res. 17, 429–441.

Tommerup, I.C., 1982. Airstream fractionation of vesicular-arbuscular mycorrhizal fungi: concentration and enumeration of propagules. Appl. Environ. Microbiol. 44, 533–539.

Tommerup, I.C., 1983. Spore dormancy in vesicular-arbuscular mycorrhizal fungi. Trans. Br. Mycol. Soc. 81, 37–45.

Trappe, J.M., 1982. Synoptic key to the genera and species of zygomycetous mycorrhizal fungi. Phytopathology 72, 1102–1108.

Trufem, S.F.B., 1990. Aspects ecologicos de fungos micrαrrizicos vesiculo-arbusculares da mata tropical umida da Ilha do Cardoso, Sp, Brazil. Acta Bot. Bras. 4, 31–45.

Trufem, S.F.B., 1995. Ecological aspects of arbuscular mycorrhizal fungi from coastal sand dunes community of Ilha do Cardoso, SP, Brazil. Revta Brasil Bot. 18, 51–60.

Verkade, S.D., 1988. Use of colloidal silica solution in the isolation of spores of *Glomus mosseae*. Mycologica 80, 109–110.

Vilarino, A., Arines, J., 1990. An instrumental modification of Gerdemann and Nicolson's method for extracting VAM fungal spores from soil samples. Plant Soil 121, 211–216.

Vivekanandan, M., Fixen, P.E., 1991. Cropping systems effects on mycorrhizal colonization, early growth, and phosphorus uptake of corn. Soil Sci. Soc. Am. J. 55, 136–140.

Walker, C., 1983. Taxonomic concepts in the Endogonaceae: Spore wall concepts in species descriptions. Mycotaxon 8, 443–455.

Walker, C., Sanders, F.E., 1986. Taxonomic concepts in the Endogonaceae III. The separation of *Scutellospora* gen. nov. from *Gigaspora* Ger. and Trappe. Mycotaxon 27, 169–182.

Wright, S.F., Upadhyaya, A., 1996. Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. Soil Sci. 161, 575–586.

Wright, S.F., Morton, J.B., Sworobuck, J.E., 1987. Identification of a vesicular-arbuscular mycorrhizal fungus by using monoclonal antibodies in an enzyme-linked immunosorbent assay. Appl. Environ. Microbiol. 53, 2222–2225.

Wright, S.F., Franke-Snyder, M., Morton, J.B., Upadhyaya, A., 1996. Time-course study and partial characterization of a protein on hyphae of arbuscular mycorrhizal fungi during active colonization of roots. Plant Soil 181, 193–203.

Wu, C.-G., Chen, Z.-C., 1986. The Endogonaceae of Taiwan I. A preliminary investigation on Endogonaceae of bamboo vegetation at Chi-Tou areas, central Taiwan. Taiwania 31, 65–88.

Zajicek, J.M., Hetrick, B.A.D., Owensby, C.E., 1986. The influence of soil depth on mycorrhizal colonization of forbes in the tallgrass prairie. Mycologia 78, 316–320.